Unit 3: The Microenvironment, its role in regulating stem cell fate, and Cancer

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California State Standards:

1.a. Students know cells are enclosed within semipermeable membranes that regulate their interaction with their surroundings.

4. Genes are a set of instructions encoded in the DNA sequence of each organism that specify the sequence of amino acids in proteins characteristic of that organism. As a basis for understanding this concept:

a. Students know the general pathway by which ribosomes synthesize proteins, using tRNAs to translate genetic information in mRNA.

"After learning about transcription and translation through careful study of expository texts, students can simulate the processes on paper or with representative models."

b. Students know how to apply the genetic coding rules to predict the sequence of amino acids from a sequence of codons in RNA.

"In eukaryotes, the initial RNA transcript, while in the nucleus, is composed of exons, sequences of nucleotides that carry useful information for protein synthesis, and introns, sequences that do not. Before leaving the nucleus, the initial transcript is processed to remove introns and splice exons together. The processed transcript, then properly called mRNA and carrying the appropriate codon sequence for a protein, is transported from the nucleus to the ribosome for translation."

d. Students know specialization of cells in multicellular organisms is usually due to different patterns of gene expression rather than to differences of the genes themselves.

"Nearly all cells in an organism contain the same DNA, but each cell transcribes only that portion of DNA containing the genetic information for proteins required at that specific time by that specific cell. The remainder of the DNA is not expressed. Specific types of cells may produce specific proteins unique to that type of cell."

5.b. Students know how to apply base-pairing rules to explain precise copying of DNA during semiconservative replication and transcription of information from DNA into mRNA.

Goals:

- Understand that genes are a set of instructions, encoded by the DNA sequence, that denote the types and sequence of amino acids during protein synthesis.
- Demonstrate the central dogma of biology by modeling the transcription of a DNA sequence to mRNA and the translation of mRNA codon triplets into amino acids that fold to form a protein.

- Explain the significance of signals from the microenvironment and gene expression in the formation of specialized cells in multi-cellular organisms.

Objectives:

- SWBAT define *genotype, phenotype* and *microenvironment* in terms of cell fate decision and how a cell is able to specialize appropriately.
- SWBAT differentiate between genotype and gene expression in terms of protein synthesis (which results in a phenotype.)
- SWBAT explain the relationship between DNA, mRNA, and tRNA as well as the processes used to form a protein during protein synthesis.
- SWBAT simulate the production of different types of mature cells from an adult stem cell depending on signals from the microenvironment using the framework of the central dogma of biology.
    - AP students can begin to understand the limitations of the central dogma, in that RNA often regulates gene expression
- SWBAT recognize factors in the microenvironment which control gene expression and cell fate, including signaling proteins, extracellular matrix proteins, forces, and cell-to-cell interactions.
- SWBAT describe how factors in the microenvironment signal stem cells to proliferate or differentiate (example mammary gland progenitor cell).
- SWBAT investigate applications of appropriate cell response to signaling in topics such as colon stem cells and hair follicles while exploring how aberrant gene expression could *but does not always* lead to cancer.
- SWBAT simulate a reductionist method to understanding the role of the microenvironment by simulating array systems and by modeling a Microenvironment Array.

Background information:

What is "cell fate?"

A regular, non-cancerous adult stem cell is for most of its lifetime **quiescent** or quiet—suspended in **G0** and at baseline metabolic activity levels. The stem cell is activated by signals from the immediate outside environment. This puts into motion pathways (**signaling cascades**) eventually leading to selective gene transcription and a shift in cell behavior or phenotype. When such a shift occurs, it is believed a significant "cell fate" decision has been made. Such a change could include the decision to **proliferate** and **differentiate** into a more mature cell type. For example, under certain signals, a neural stem cell may shift towards a neuronal fate instead of a **glial cell** fate. Different stem cell types vary in the number of possible cell fate decisions, often described as their potency. Adult stem cells are more restricted in which types of mature cells they can become (they are less potent) than embryonic stem cells, which can turn into any of the body's 200+ cell types. Adult stem cells are multipotent; they may

eventually become more than one mature cell type, filling the tissue's need for new cells. If every cell is genetically identical, how are there different stem cell types? Moreover, with all the possible cell fate decisions before them, how does a stem cell choose a given path over another?

If every cell in an organism has the same DNA sequence, why aren't all cells the same?

Cell phenotype depends on context-specific cues within tissues. Even though each cell in your body has the same DNA sequence, composed of **exons** and **introns**, different sets of genes are expressed in different tissues. Each tissue has a different **microenvironment** that interacts with each cell. As will later be explained in detail, the microenvironment consists of signals that in various combinations influence cell behavior. Cells have the arduous task of integrating multiple extracellular signals and attempting to perform the desired response(s).

A stem cell fate decision towards a certain mature cell phenotype depends on **gene expression** changes resulting in the production (**upregulation**) or inhibition (**downregulation**) of proteins. Increasing or decreasing the amount of various proteins initiates functional cellular changes that in some cases completely transform the cell type.

It will be helpful to review with your students how genes are expressed into proteins at the molecular level so they can have the foundation to understand the more complex phenotype shift of a differentiating stem cell. You may wish to teach students about protein production through the example of **hematopoietic** (blood-forming) **stem cells.** These cells can receive an outside signal or set of signals, which are "passed along" by surface receptors, plasma membrane proteins, and cytoplasmic **enzymes** into the nucleus, where **transcription factors** bind to the promoter regions of genes to control their activation or deactivation. Transcription factors are very important in controlling stem cell phenotype; in many cases these factors act to keep a stem cell as a stem cell (in other words, to keep it from differentiating) by suppressing production of proteins and promoting others.

In the Invitation portion of this unit, the student activity helps explain to your students that outside signals, mediated by intracellular transcription factors, result in gene expression changes important for a blood stem cell to specialize into a white blood cell or a red blood cell. Exposure of blood stem cells to a different **signaling factor** or set of factors could alter the cell fate. Your students can simulate the differentiation process from a blood progenitor cell (**common myeloid progenitor**) into either red or white blood cells, depending on the identity of the transcription factor to which each student's "cells" are exposed. You provide them with *either* Erythropoietin *or* Colony Stimulating Factor, and the students edit their raw "partial chromosome" transcript containing two gene sequences. This models transcription and translation resulting from gene activation by one of the factors, leading to either a RED or WHITE protein (capital letters spell the amino acid sequences). This activity uses a free downloadable program called

Another Plasmid Editor (ApE), normally used to search within and design bacterial plasmids for genetic engineering, and that has a basic interface where students can manipulate the DNA transcript.
For a good review of transcription and translation, we recommend students color and work through these handouts from The Biology Corner:
http://www.biologycorner.com/worksheets/DNAcontrols.html and
http://www.biologycorner.com/worksheets/trans_coloring.html.
During transcription, the synthesis of single-stranded, messenger RNA from a DNA template strand in the nucleus includes cutting out introns ("junk DNA") and splicing together exons that code for parts of the protein. Then, the entire coding strand of mRNA exits the nucleus and interacts with ribosomes and tRNA, which use the mRNA's codon triplets as a guide to add amino acids to the growing peptide. In addition to these roles in transcription and translation, RNA also controls the expression of genes. During RNA interference (RNAi), a natural process that has been adapted for use in molecular biology, small double-stranded RNAs target their matching-sequence mRNAs for destruction by RNAi machinery. This leads to gene silencing, useful as a method for temporarily suppressing specific proteins in eukaryotic cells. For more detail on this process and animations, visit http://www.nature.com/focus/rnai/animations/index.html. These animations may be helpful for your background knowledge or for more advanced students as an introduction to the BioBridge RNAi lab, available for teachers in the San Diego area.

The Microenvironment
It is increasingly becoming known that cell interactions with the microenvironment can heavily influence their behavior. Students can think of the microenvironment as the dorm in which a stem cell lives and functions. The microenvironment has non-living materials (concrete, rebar, furniture), other cells (roommates, neighbors, and visitors), as well as signaling molecules (voices, visuals, objects in the way) that help our stem cell character navigate in the world and decide what to do. For cells, even forces (sensed as physical variations in the extracellular matrix) can influence cell fate. A cell's microenvironment is its local interface with the outside world and feeds into its behavior. The specific microenvironment of stem cells is called the **stem cell niche** (*neesh*)*;* this environment influences the development of stem cells from quiescence through stages of differentiation. Just like the ecological niche of an organism, a stem cell niche is unique to the individual or small population and guides its dynamics. Here are the four major components of the microenvironment.

Soluble factors
Received from the extracellular environment, soluble factors typically bind a cell's plasma membrane or cytoplasmic receptors. While soluble factors, such as proteins, hormones, and cytokines, vary in biochemical composition and origin, their signaling outcomes can be classified into four main cell behaviors.

- Survival: Typically achieved through suppressing apoptosis.
- Division: Promotes synthesis of proteins and inhibits their degradation. Also can relieve intracellular blocking of cell cycle.
- Differentiation: Cell specialization occurs through changes in metabolism, gene expression, and cell shape/migration.
- Death: In most cases, an intracellular proteolytic pathway is activated and the bulk of the work performed by a certain class of proteins called caspases.

Granulocyte colony-stimulating factor (G-CSF) is an example of a cytokine that activates precursors in the bone marrow to produce mature granulocytes, a type of white blood cell. A soluble factor's effect on behavior can differ by cell type. G-CSF acts as a migration signal to hematopoietic stem cells, inducing their release into the blood stream without affecting their fate. In the central nervous system, G-CSF also stimulates the differentiation of new neurons from neural precursor cells.

Cell-cell interactions

Cells can also receive extracellular signals from connections made with their neighbors. These connections can vary in size, composition, strength, and cargo transported. Below is a list of major cell junction types.

- Tight junctions: As the name implies, these seal cells together, forming a molecule-impermeable sheet and localizing different integral membrane proteins to either side. Epithelial tissues like the intestines require a strong barrier for bidirectional transport.
- Anchoring junctions: The two main types are adherens and desmosome junctions. Adherens junctions are narrow bands or patches built from **cadherins** and **catenins** that provide robust mechanical attachments between cells. For example, adherens junctions help cardiac cells stay together despite constant beating of the heart. Desmosomes are small patches attaching cells that link to intermediate keratin filaments in the cytoskeleton, which provide a framework of great tensile strength.
- Gap junctions: Consisting of cylindrical channels constructed from connexins, gap junctions allow passage of small molecules to and from adjacent cells. These junctions serve diverse purposes, from synchronizing heart muscle cell contraction to coordinating neural tube formation during embryogenesis.

Cell adhesion molecules do more than hold cells together. They also act as receptors that transduce signals controlling contact-mediated growth suppression and differentiation. Normal cells grown in a petri dish will divide until they completely cover the surface, when they experience **contact inhibition** and stop dividing. But cancer cells lose this contact inhibition, suggested by the observation that they continue growing on top of each other after they cover the whole petri dish. Cancer cells do not respond normally to growth-regulatory signals induced by cell-cell interactions. Carcinomas are cancerous epithelial growths in which the cells have lost their desmosomes, leading to metastasis. Also, in several developmental systems, cell-cell contact is important in determining cell fate through interactions of specific cell surface receptors on neighboring cells. Another function of cell adhesion molecules, in combination with cytoskeletal proteins, is maintenance of cell shape. Cell shape can be experimentally altered by coating different thicknesses of polyHEMA (a hydrogel polymer used for growing cells) onto plastic petri dishes. It has been shown that cells plated on a thick layer of polyHEMA are spherical and have reduced DNA synthesis (they do not enter S-phase) as compared to cells of the same type grown on a thinner layer, which become flatter and are able to enter S-phase and divide. For more information about and examples of each of these junctions, visit http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/J/Junctions.html.

Extracellular matrix proteins

These proteins make up the extracellular scaffold within which cells adhere and grow. Extracellular matrices are made of collagen, fibronectin, laminin, and other proteins produced by connective tissue cells. They all have unique structures that contribute to both the biochemical and mechanical signaling in a given tissue.

- Collagen is a triple helix and occurs in many places throughout the body. 29 types of collagen have been identified. Over 90% of the collagen in the body belong to types I, II, III, and IV.

  Collagen I: Skin, tendon, vascular, ligature, organs, bone (main component of bone)
  Collagen II: Cartilage (main component of cartilage)
  Collagen III: Reticulate (main component of reticular fibers), commonly found alongside type I.
  Collagen IV: Basement membranes
  Collagen V: Cells surfaces, hair and placenta

- Fibronectin is a **dimer** present as a soluble protein in the blood (a liquid matrix for blood cells) and as an insoluble component of the extracellular matrices in our bodies. Fibronectin plays a role in cell adhesion, growth, migration and differentiation, and it is important in embryonic development and wound healing. In embryonic development, fibronectin guides

migration and cell attachment and without it, mesodermal, neural tissue, and vascular tissue do not develop normally. In wound healing, plasma fibronectin and fibrin are deposited and form a clot. **Fibroblasts** bind to it and replace the bound plasma fibronectin with new extracellular fibronectin in amounts matching the surrounding tissue. The breaking down of fibronectin thus promotes contraction of the surrounding tissue extracellular matrix and healing of the wound. Abnormal expression of fibronectin is implicated in lung cancer and tumor cell morphology.

- Laminin is featured (along with collagen IV) in the basement membrane of tissues as its cross-like structure allows the short arms of laminin molecules to link into sheets and the long-arms to attach cells. Cellular **integrins** mediate this attachment and signaling. Laminins can control directly or indirectly cellular activities such as adhesion, migration, differentiation, polarity, proliferation, apoptosis, and gene expression. It is important in branching morphogenesis of the lung, kidney, breast, and salivary gland, where cells clump together and protrude to form glands and ducts. It is expressed in the central and peripheral nervous systems, notably by Schwann cells which insulate PNS neurons with myelin (oligodendrocytes myelinate neurons in the CNS.) Laminin heavily influences Schwann cell proliferation, differentiation, and survival during PNS development. Mutations in laminin causing decreased nerve insulation and other effects are associated with the symptoms of Muscular Dystrophy.

Forces

The last section described collagen, fibronectin, and laminin as proteins that can assemble into extracellular matrices in the body and signal to cells. These proteins plus adherent cells form a matrix that varies in stiffness, the degree to which a material can maintain its original form under force, relative to its density and organization. Because of this, tissues differ in stiffness; for example, brain is soft and very elastic, skin has intermediate stiffness, muscle is stiffer, and bone is very stiff and inelastic. Cells can sense the stiffness of their surroundings by anchoring and pulling using myosin-based contraction and adhesion molecules (like integrins and cadherins). This pulling also generates small contractile forces that can change the organization of the extracellular matrix. Cells can respond to the stiffness they feel by reorganizing their cytoskeletons and initiating other cellular processes. In these ways, there is a dynamic, reciprocal relationship between the cells and the extracellular matrix with regards to forces felt and applied. Put differently, when the matrix imposes forces on cells, cells feel these forces, and then they respond by changing their own stiffness—sometimes even locally reorganizing the matrix to change its stiffness, which can signal a different response from the cells!

Research on how stem cells sense and respond to forces in the microenvironment has implications for tissue engineering. When creating a scaffold for cell transplantation, knowing exactly how the organization of the engineered matrix will influence cell behavior and vice versa could mean the difference between a successful transplant and rejection. A scaffold usually refers to a 3-dimensional gel (made similarly to Jell-O) consisting of matrix proteins like collagen or synthetic polymers, although a scaffold can also refer to an organ that has been treated with chemicals to remove all cells, with just the extracellular matrix left in the shape of the organ. (See a great article on decellularizing rat and pig hearts to treat heart disease. http://www.technologyreview.com/Biotech/20058/) A researcher embeds cells inside the scaffold and bathes it in growth media and factors that promote survival. To a stem cell, being in "3D" is a drastically different experience than growing in "2D" on top of a petri dish; the stiffness of the surface, among other things, influences cell shape, growth, and responses to signaling molecules. Because of this, cell biologists should carefully consider how the surface on/in which their cells are grown plays into the phenomenon they are studying.

Varying substrate stiffness can even influence stem cell fate. Mesenchymal stem cells—multipotent cells found in the bone marrow that naturally differentiate into bone, cartilage, fat, tendon, muscle, and marrow stroma—can be isolated and propagated in culture. A study from the University of Pennsylvania showed that mesenchymal stem cells commit to different lineages depending on the stiffness of the substrate. When grown on a collagen gel that mimics the stiffness of brain tissue, the mesenchymal stem cells differentiate into neurons! Grown on a 10-fold stiffer gel, reminiscent of muscle tissue, the stem cells turn into muscle cell precursors. Finally, when grown on a very stiff gel, the stem cells differentiate into bone cells. In addition to these observations, the researchers pinpointed the mechanism of the stem cell response. Using a drug called blebbistatin, they inhibited nonmuscle myosin II—involved in sensing matrix elasticity—in the stem cells. Without the action of nonmuscle myosin II, the cells could not differentiate according to the stiffness.

Current research on the microenvironment

Research on how cell behavior and the microenvironment are related is progressing rapidly. We can maintain one cell type for long periods of time (proliferation), differentiate stem cells into mature progeny, and induce cells to perform other cell behaviors in a dish because we have identified and applied the necessary and sufficient microenvironmental factors that direct cells in these ways. By identifying powerful factors characteristic of the abnormal microenvironment—one that allows cancer to grow, for example—we will know much more about diseases and potentially how to stop them. Cell culture is an example of "outside → in" phenotype control because we are using factors outside of the cell to induce gene expression changes or to control cells to stay the same.

How do we identify factors outside the cell that influence cell behavior? There are many possible combinations of microenvironmental factors to which cells can respond, so we would need a way to test a large number of possibilities. A tool called the Microenvironment Array (MEArray) helps researchers test thousands of individual microenvironments on stem cells to observe how they respond. In the Application portion of this unit, students will learn about Mark LaBarge and Mina Bissell, who narrowed down the components of the breast microenvironment required for mammary gland progenitor cells to differentiate into myoepithelial cells or luminal epithelial cells.

On the other hand, "inside → out" phenotype control is to manipulate the genomic sequence using recombinant DNA technology, causing a phenotype shift or change in cell behavior. This enables us to perform dramatic manipulations of cell fate—to the point of being able to reprogram skin cells into stem cells, and one fully differentiated cell into another. Being able to turn one cell type into another is dependent on the introduced genes' expression inside the cell, guided by the correct signals from the new, simulated microenvironment outside.
You may want to teach students about different types of arrays as examples of technologies used to see what is going on inside cells and how they change behavior. Another type of array, called a cDNA array, compares gene expression profiles of different cell types or disease states. High-throughput cDNA arrays give us a huge amount of information about what's happening inside cells as they change phenotype and are used widely in biology. This technique is described clearly in an interactive animation you can do with your students, found here: http://www.bio.davidson.edu/Courses/genomics/chip/chip.html.

Focus on Cancer
In cancer, the genomic sequence is mutated, causing problems with the produced proteins. This results in cells with a cancerous phenotype, which can be considered an "inside-out" phenotype shift. Some examples of cancer phenotypes and causes (carcinogens such as viruses, toxins, and UV damage) are explained in the Supplementary PowerPoint presentation (cirm.ca.gov/curriculum_unit-3) for students. While reading this section and teaching about the causes of cancer, try to brainstorm answers to these questions:

- Is a genetic mutation necessary for cancer?
- Could cancer occur only by manipulating the microenvironment?
- If you took a piece of normal tissue and inserted it inside a tumor, what would happen?
- If you infect a chicken embryo with a cancer-causing virus, and the chicken grew up cancer-free, would you assume the chicken's cells were noncancerous?

- How might we test ways to see if certain microenvironments can stop cancer from growing?

What causes cancer?
Cancer is an abnormal cell phenotype characterized by uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues), and sometimes **metastasis** (spread to other locations in the body via lymph or blood). For a cell to turn malignant—highly cancerous—multiple mutations in important regulatory genes must accumulate. Generally speaking, mutations in two basic classes of genes—**proto-oncogenes** and **tumor suppressor genes**—are what lead to cancer.
Tumor Suppressor Genes
Tumor suppressor genes are vital for stopping a cell or group of cells from spontaneously dividing without obtaining the correct signals for initiation or continuation of the cell cycle (=growth). Tumor suppressor genes are normal genes that slow down cell division, repair DNA mistakes, and tell cells when to die (a process known as **apoptosis** or programmed cell death). So, a mutated tumor suppressor gene leads to cancer because cells make an abnormal protein that doesn't correctly act to suppress "cancerous attributes" like rapid cell growth, survival, and metastasis. Many different tumor suppressor genes have been identified, including p53, BRCA1, BRCA2, APC, and RB1 (ACS, http://www.cancer.org/docroot/ETO/content/ETO_1_4x_oncogenes_and_tumor_suppressor_genes.asp). An example pathway of a specific tumor suppressor is provided below for discussion with advanced or AP students.

Oncogenes
Mutations in proto-oncogenes, which normally code for proteins regulating cell growth, **mitosis**, and differentiation, can lead to their abnormal or overactive function—also leading to cancer. Once a proto-oncogene has been mutated, it is called an **oncogene**, the expression of which contributes to a cancerous phenotype. Oncogenes cause some sort of interference with or acceleration of the cell cycle. They tend to be caused by radiation, toxic compounds, or some biological stress, which degrades the gene or mutates it, thus changing its normal function. Transcription and translation of an oncogene can have a devastating effect on other cellular functions either by affecting the amount of protein produced, the movement of nucleic DNA (transposons), or by causing another **point mutation** in another proto-oncogene. Other causes of oncogene activations include chromosomes that have broken and rejoined incorrectly, and **translocation** of gene fragments from one chromosome to another. If a translocated oncogene ends up near an active promoter (or other transcriptional control element), its activity may increase, making it an oncogene.

Specific examples of Tumor suppressor genes: Cell cycle regulators

Cyclin dependent kinases and inhibitors are cell cycle regulator proteins that can be the targets of cancer-causing mutations. For example, **p53** controls key elements in the cell cycle. The expression of p53 is activated by damaged DNA somewhere else in the genome. If the cell were to continue dividing, a DNA replication error, possibly a serious one, would be made and passed along to the daughter cells. P53 acts to halt the cell cycle so the broken DNA can be successfully repaired before division.
Once *p53* is produced, it acts as a transcription factor, activating **p21.** This gene stops the cell's growth by creating an inhibitory protein (*p21*) that binds to **cyclin-dependent kinases** A & B. Cyclin-dependent kinases normally bind to cyclin, setting off signaling pathways enabling cell division to begin. **Cyclin-dependent kinase inhibitors** such as *p21* bind to and inactivate the cyclin-dependent kinase/cyclin complex, halting cell division before S-phase. *p53* then activates DNA repair genes to fix the damaged DNA. If repair is successful, the cell will continue past the G1 checkpoint into S-phase and divide. If repair is impossible, *p53* activates a suicide gene which prompts **lysosomes** to hydrolyze (dissolve) the cell—apoptosis. If *p53* doesn't function correctly, these regulatory cycles cannot occur and the cell can become cancerous. In fact, in 50% of cancers *p53* is missing or mutated.

Cancer stem cell hypothesis
A tumor is a collection of cell types; some are terminally differentiated, some can proliferate and differentiate to some degree, and a significant number may be able to form all tumor cell types—called **cancer stem cells.** These cells give rise to additional tumors when transplanted to another animal, and those tumors contain all cell types in the original tumor. The cancer stem cell hypothesis argues that mutations to stem cells activate them into cancer stem cells, which cause tumors and retain the capacity to differentiate into new tumors. When different cell types in a mouse tumor were dissociated then sorted into populations of similar types, only one small population was able to give rise to new tumors when injected into different immune-deficient mice. This implies there could be a population of "adult stem cells gone wrong"—that have gained the ability to proliferate and differentiate into multiple tumor cell types—responsible for the initiation of the cancer.
For a background article (appropriate for AP level classes) on the cancer stem cell hypothesis, and how the microenvironment may plays a role in cancer, see:
http://www.genengnews.com/articles/chitem.aspx?aid=2130&chid=4

The Microenvironment and Cancer
The four aspects of the microenvironment—soluble factors, extracellular matrix proteins, forces, and cell-cell interactions—are implicated in cancerous responses of cells. As discussed earlier, cancer arises from DNA mutations in a cell that cause proliferation, differentiation, and metastasis (migration and spreading). Could cancer arise from a normal stem cell in the wrong microenvironment? When embryonic stem cells (pluripotent, and incredibly similar in their characteristics to immortalized cancer cell

lines) are injected into immune-system-deficient mice (**SCID mice**), so that the immune system cannot fight the stem cells, these mice form tumors that contain cell types from all three germ layers, as a Pluripotent cell would be expected. Here, the embryonic stem cells suddenly were moved to a wildly different microenvironment than embryonic stem cells need to stay the same. This barrage of abnormal differentiation cues to the pluripotent stem cells caused them to turn into many different cell types.

Like the above example, adult stem cells or other cells exposed to abnormal cues might be expected to turn cancerous, in combination with or even without cancer-causing mutations to their genomes. Mutations in surrounding cells, or something else irregular about the niche, might push a cell toward a cancer phenotype.

It would be therapeutically useful to know if a normal microenvironment could cause inhibition of a cancer cell. Early studies with **embryonal carcinoma cells**, which are undifferentiated cancer cells that form different cell types when introduced into adult mice, showed that if injected into a **blastocyst**, the cells that were derived from the cancer cells no longer caused cancer in the mice that developed from the injected blastocyst. This suggested that the microenvironment prevented or reversed the cancerous properties, then caused them to develop normally in the presence of cues from the normal blastocyst environment.

Another study supporting the idea that cancer cells require additional signals besides genetic abnormalities to become cancerous used a cancer-causing virus that required a certain microenvironment for tumor growth. The Rous Sarcoma Virus (RSV) caused **sarcomas** (cancerous growth of the connective tissue) in mature chickens at the site of injection. Scientists observed that when the virus was injected into chick embryos, it did not cause cancer even though the chick cells derived from those embryos had the ability to gain cancerous properties or “transform” when cultured **in vitro**. Surprisingly, a later study showed that creating a lesion or wound would cause the surrounding cells to turn cancerous and cause tumors. More specifically, it was shown that a protein called TGF-beta, involved in wound healing, was sufficient to induce tumor formation. These findings indicated that factors involved in wound healing could promote transformation of cells and that this particular microenvironment provided important signals for the growth of cancer.

More resources on breast cancer development

Diagram http://www.nature.com/labinvest/journal/v88/n5/fig_tab/labinvest200814f1.html

Paper http://www.nature.com/labinvest/journal/v88/n5/full/labinvest200814a.html

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Glossary

Adherens junctions - appear as bands encircling the cell (zonula adherens) or as spots of attachment to the extracellular matrix (adhesion plaques). Cadherin “hooks” fit together on the outside of the plasma membranes with the catenin portions entering inside on the cytoplasmic face, attaching to the actin cytoskeleton.

Apoptosis - programmed cell death

Blastocyst - 5-14 day old embryo; the structure formed after the morula; it consists of about 100 cells, with pluripotent stem cells located in the inner cell mass

Cadherins - hook-like proteins on the plasma membranes of cells that serve to link cells together as part of an adherens junction.

Cancer stem cell - a mutated stem cell, found in tumors, which can differentiate into all cells in a tumor

Cancer stem cell hypothesis – a hypothesis that states some cancers are initiated and propagated by cancer stem cells, or stem cells that have been mutated or subjected to an abnormal microenvironment

Catenins – the cytoplasmic portion of an adherens junction; these proteins (complexed with cadherins) bind the actin cytoskeleton to secure cells.

Clonal evolution model - a cell (clone) with a mutation in a tumor suppressor gene or oncogene will expand only if that mutation gives the clone a competitive advantage over the other clones and normal cells in its microenvironment. Thus, the process of carcinogenesis is a process of Darwinian evolution, known as somatic or clonal evolution.

Common myeloid progenitor - cells which give rise to “megakaryocyte/erythrocyte [Red Blood Cell] or granulocyte/macrophage [White Blood Cell] progenitors”, which will later give rise to RBC’s or White Blood Cells (http://www.ncbi.nlm.nih.gov/pubmed/10724173)

Contact inhibition - the natural process of arresting cell growth when two or more cells come into contact with each other. This property is used to distinguish between normal and cancerous cells.

Cyclin-dependent kinases - protein kinases that control cell cycle progression in all eukaryotes and require physical association with cyclins to achieve full enzymatic activity.

Desmosomes - a cell structure specialized for cell-to-cell adhesion; localized in patches randomly arranged on the sides of plasma membranes; help to resist shearing forces; bind muscles cells to one another.

Differentiate - the transformation of an unspecific cell to a cell with a specified role in the microenvironment.

Dimer - a chemical or biological entity consisting of two structurally similar subunits called monomers, which are joined by non-covalent bonds, which can be strong or weak.

Downregulation -

Embryonal carcinoma cell – a type of cancer cell which has the ability to switch fates with embryonic stem cells (and vice versa) depending on the microenvironment and genetic reprogramming

Enzymes - proteins that catalyze (increase the rates of) chemical reactions. In enzymatic reactions, the molecules at the beginning of the process are called substrates, and the enzyme converts them into different molecules, called the products.

Exon - a nucleic acid sequence that is represented in the mature form of an RNA molecule (messenger RNA) after non-coding RNA (intron) removal.

Factor - any ligand, transcription factor, chemical, or microenvironmental "thing" that elicits a reaction from the cell

Fibroblast - a cell found in connective tissue that produces fibers such as collagen

G0 – (pronounced G zero) pre-mitosis, resting state, or quiescence. Most cells are usually 'standing by' in this phase before signals cause them to undergo mitosis. A tissue stem cell exists in G0 until it is activated.

Gap junctions - directly connect the cytoplasm of two cells, which allows various molecules and ions to pass freely between cells.

Gene expression - the process by which DNA is transcribed into mRNA, then translated into protein.

Glial cell - Cells located in the CNS which offer support and insulation to neurons; they are the most abundant type of cell in the CNS, with three types: astrocytes, oligodendrocytes, and microglia

Hematopoietic (blood-forming) **stem cell -** a stem cell of the bone which gives rise to Red Blood Cells, other myeloid cells, and lymphoid cells

In vitro - performed in a test tube or culture, outside of a host organism

Integrin - receptors that mediate attachment between a cell and the tissues surrounding it, which may be other cells or the extracellular matrix; play a role in cell signaling thereby regulating the cell cycle and defining cellular shape and mobility.

Intron - a DNA region within a gene that is not translated into protein.

Ligands – a substance that binds to and forms a complex with a receptor or target protein; often triggers a signal from the cell.

Lysosome - vesicles filled with hydrolytic enzymes which are used to break down organic matter in the cell, or the cell itself if during apoptosis

Malignancy (malignant) - tumor causing cancer which is liable to spread to different parts of the body, opposite from benign

Metastasis - spread of cancer to other locations in the body via lymph or blood

Microenvironment – a cell's interface with the outside world, which feeds into its behavior through gene expression; soluble factors, extracellular matrix molecules, cell-cell contacts, and forces are components of the microenvironment.

Oncogene - a mutated proto-oncogene, which normally codes for proteins regulating cell growth, mitosis, and differentiation, is called an oncogene. An oncogene is said to be activated, which means the gene has an abnormal or overactive function, causing the cell to transform into cancer.

p21 – a cyclin-dependent kinase inhibitor gene; it encodes for the protein WAF1, which binds to and inhibits the activity of cyclin-dependent kinases, prevalent in G1 of the cell cycle. It is a cell cycle regulatory gene.

p53 - the "Guardian Angel" of the cell, this gene regulates the production of other proteins and the transcription factors needed to express other regulatory genes; it activates DNA repairing enzymes/proteins and apoptosis if the DNA cannot be repaired.

Point mutation – a single nucleotide mutation; can be in the form of a deletion or insertion

Proliferate - to grow into larger numbers, or multiply, by cell division

Proto-oncogene – a gene that normally codes for a protein regulating cell growth, mitosis, or differentiation, which when mutated, can lead to abnormal or overactive function and cancer. Once a proto-oncogene has been mutated, it is called an oncogene.

Quiescent - dormant, as in the G0 phase; describes a stem cell that still has basal metabolic activity but behaviorally it is staying still in one spot, not proliferating, not differentiating, simply surviving.

Sarcoma – cancerous growth of connective tissue; differs from carcinomas, which are cancers of the epithelium

SCID mice - Severe Combined Immunodeficiency, a condition which means that the immune system is unable to fight any disease or infection. SCID mice are used to observe the effects of viruses/bacteria/cancer causing factors, and medicines to fight said abnormalities

Signaling cascade - signal transduction refers to any process by which a cell converts one kind of signal or stimulus into another; most processes of signal transduction involve ordered sequences of biochemical reactions inside the cell, which are carried out by enzymes and second messengers, resulting in a *signal transduction pathway*. The number of proteins participating in signal transduction increases as the process emanates from the initial stimulus, resulting in a signaling cascade, beginning with a relatively small stimulus that elicits a large response. This is referred to as *amplification of the signal*.

Signaling factor – any molecule that elicits a signaling pathway or cascade.

Stem cell niche - the microenvironment in which stem cells are found that controls their behavior

Tight junctions - closely associated areas of two vertebrate cells, in which the membranes join to form a virtually impermeable barrier to fluid and ions.

Transcription factor - a type of protein which binds to a specific sequence on DNA and signals/aids RNA polymerase to start transcription

Translocation - the movement of DNA/ chromosomes from its original spot to another; this often results in deleterious mutations.

Tumor - a cancerous mass which contains a collection of cell types; some are terminally differentiated, some can proliferate and differentiate to some degree, and a significant number may be able to form all tumor cell types.

Tumor suppressor gene - normal genes that slow down cell division, repair DNA mistakes, and tell cells when to die. A mutated tumor suppressor gene leads to cancer because cells make an abnormal protein that doesn't correctly act to suppress cancerous attributes like rapid cell growth, survival, and metastasis.

Upregulation - the process by which a cell increases the quantity of RNA or protein in response to a signal.

Outline of Unit

Invitation

1. Show animation: Colony Stimulating Factor (Drew Berry and Etsuko Uno, 2009) http://www.wehi.edu.au/education/wehi-tv/?page=3

   Synopsis of animation: When the immune system recognizes a pathogenic invasion, immune cells respond by releasing colony stimulating factors (CSF), which stimulate stem cells in the bone marrow to construct specialized white blood cells. These white blood cells begin to form a colony, which will continue to undergo cell division. As the colony grows and matures, the formation of an immune cell army then travels through the circulatory system with a mission to eradicate the infection. Since their discovery, colony stimulating factors have helped millions of cancer patients survive destruction to the bone marrow that often results from high-dose chemotherapy.

2. For an introduction or review of the genetic processes underlying the above proliferation, show these animations on transcription and translation and ask the student WHY these processes are important to the effects of Colony Stimulating Factor on bone marrow stem cells.

   a. Navigate to http://www.wehi.edu.au/education/wehi-tv/?page=2, then scroll down to the 3rd and 4th animations displayed on the page. These can be played through your browser window by clicking play or can be downloaded individually by clicking "Download the Animation"

      1. DNA Central Dogma Part 1 Transcription (Drew Berry, 2003)
      2. DNA Central Dogma Part 2 Translation (Drew Berry, 2003)

   b. Have students practice transcription and translation using the following interactive animation.
      http://learn.genetics.utah.edu/content/begin/dna/transcribe/

3. **AP Extension** Activity: How can a cell with one genotype give rise to cells with different phenotypes?

   i. Choices of a Common Myeloid Progenitor cell

      1. Become erythrocytes (red blood cells)
      2. Become granulocytes (white blood cells)

ii. Use "From Genotype to Phenotypes: Red Blood Cell or White Blood Cell?" handout for the following exercise (Appendix A, http://www.cirm.ca.gov/files/Education_Portal/Unit-3/Unit_3_Appendix_A.pdf)

iii. In this exercise, your students will simulate the production of EITHER red blood cells OR white blood cells from common myeloid progenitor cells. After receiving soluble factor (signaling molecules) a) Erythropoietin OR b) Granulocyte-Colony Stimulating Factor, students will go through the process of transcription and translation to create either a) RED protein or b) WHITE protein.

1. This activity required preparation in advance. See next page for instructions.

Instructions

This is a much simplified version of these signaling pathways, so remind your students that one signaling molecule may affect many genes (signal amplification). Producing one "red" protein will not cause a myeloid progenitor to immediately turn into an erythrocyte. In reality, many specific proteins must be expressed to influence the decision to make a red blood cell or a white blood cell. This simplified exercise demonstrates how a protein specific to one or another more specialized cell type is made from a common progenitor.

- Before students begin the activity, download program onto all student computers (or your computer if you are performing the demonstration): Another Plasmid Editor (ApE) http://www.biology.utah.edu/jorgensen/wayned/ape/
- Using this program, students can simulate the production of different types of mature cells from an adult stem cell depending on signals from the microenvironment.
- Tell students that they each have an identical copy of a short portion of the genome of a Common Myeloid Progenitor cell (CMPs). Whether daughter cells of CMPs become red blood cells or white blood cells depends on which genes are expressed (transcribed and translated into proteins that lead to different phenotypes.) This process can be dictated by factors in the extracellular environment, such as the introduction of Erythropoietin or Granulocyte-Colony Stimulating Factor.
    - Erythropoietin (Epo) and Granulocyte-Colony Stimulating Factor (G-CSF) are soluble factors that bind to their specific cell surface receptors, instigating a signaling cascade which ends with the binding of transcription factors to DNA, up- or down-regulating specific genes. For more about transcription factors and their role in hypoxia (low oxygen), as well as a list of known factors with links to pathway information, check out http://www.rndsystems.com/molecule_group.aspx?r=1&g=985.

- Note: Depending on the depth of explanation you wish to provide your students, you can discuss transcription factors or say that Epo and G-CSF jumpstart the pathway leading to transcription of target genes.

- Give students a **raw DNA transcript** file which contains (in this order): promoter region 1, a start codon (ATG), the first gene's DNA sequence including introns and exons, a stop codon (TAA), introns, promoter region 2, a start codon (ATG), the second DNA sequence including introns and exons, and another stop codon (TAA). Assign students (or have them pick) either Epo or G-CSF, which start signaling cascades leading to the expression of either gene 1 (Epo target) or gene 2 (G-CSF target). If students get Epo, start at promoter region 1. If G-CSF, start at promoter region 2. Students will then transcribe into RNA the gene downstream of their promoter region (ATG….TAA). They will then remove the introns from the RNA transcript to make mRNA (they will be told the sequences of the introns so they know what to splice out.) From the mRNA transcript, students will use a codon chart to translate the mRNA bases into the primary amino acid sequence. The amino acid sequences will spell out either "RED" (target gene 1) or "WHITE" (target gene 2) depending on which gene was transcribed.

Exploration

Cell fate and behavior

1. How does cell phenotype come from genotype?
    a. We have 30,000 genes, but only a subset are expressed for each cell type
    b. Through gene expression, transcription and translation of this subset of genes determines how a cell looks and acts (its phenotype)
        i. Phenotype: Proteins and enzymes, surface markers, cell behaviors (migration), cell fate decisions (proliferation and differentiation).
        ii. What dictates potential? Pluripotent and Multipotent SCs respond differently to signals because they express different genes. These characteristics are results of the differential expression of subsets of genes.
    c. How does a cell know what to become? In a cell fate decision, a stem cell makes a decision to behave, for example proliferate, differentiate, or migrate. Is this decision random? If not, what influences this decision? (The decisions to proliferate and differentiate don't have to be mutually exclusive. For example, Granulocyte-Colony Stimulating Factor influences *proliferation and then differentiation*)

d. Central Dogma of Biology worksheets and "signaling" factors

   i. Students can read about examples of microenvironmental factors and regulation below in 2.

   ii. Use Central Dogma Handout (Appendix B, http://cirm.ca.gov/curriculum_unit-3)

Readings

Pick several of these for students to read and understand the effects of each microenvironment component. Choose one from each of the sections in **bold**.
Ask students to consider: What controls gene expression and cell fate?
The microenvironment, which consists of: signaling factors (soluble like Colony Stimulating Factor and steroid hormones), extracellular matrix proteins, forces, and cell-cell interactions.

a. Signaling factors

   i. EASY: Protein receptor for neural cells
   http://www.sciencedaily.com/releases/2008/11/081110153621.htm

   ii. EASY: Nerve growth factor: Easy level reading
   http://www.sciencedaily.com/releases/2009/08/090828103924.htm

   iii. MID: Stat3 turns off immune system and allows cancer to form
   http://www.sciencedaily.com/releases/2009/02/090202174457.htm

   iv. MID: Protein signaling neural formation and Notch pathways
   http://www.sciencedaily.com/releases/2009/06/090630091033.htm

   v. HARD: Proteins that cause stem cells to form skin cells
   http://www.sciencedaily.com/releases/2009/09/090913134028.htm

b. Extracellular matrix proteins

   i. EASY: ECM protein that promotes muscle cell health
   http://www.sciencedaily.com/releases/2008/12/081230072244.htm

   ii. MID: ECM determines cell fate and cancer development
   http://www.sciencedaily.com/releases/2009/02/090227093558.htm

   iii. MID: Cell movement regulated between cells
   http://www.sciencedaily.com/releases/2008/10/081002172544.htm

   iv. MID: UCSD research summary of ECM role on cells
   http://ecm.ucsd.edu/Research.html

v. HARD: General overview on role of ECM
http://www.glycosan.com/why_optimize_extracellular_matrix.html

vi. HARD: Role of ECM in cell differentiation
http://www.thefreelibrary.com/Role+of+Extracellular+Matrix+Factors+in+Stem+Cell+Differentiation+...-a0141481748

c. Forces

i. EASY/press release: http://www.eurekalert.org/pub_releases/2008-09/uoc--ubb092008.php

ii. MID: Affect of mechanical forces on embryonic stem cells
http://www.physorg.com/news175093297.html

iii. MID: Cell orientation among other cells determines its division
http://www.sciencedaily.com/releases/2007/06/070619193252.htm

d. Cell-cell interactions

i. MID: Hormone receptors inside cells
http://www.sciencedaily.com/releases/2009/07/090707094706.htm

ii. HARD: Inter-cell communication
http://www.sciencedaily.com/releases/2009/09/090921091610.htm

iii. HARD: Cell–cell interaction networks regulate cell fate
http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2724979

Lab exercise

For teachers near UC San Diego, you can use the RNAi and Genetics lab kit and protocol from BioBridge @ UCSD. RNAi is an excellent way to examine the process of the central dogma. In this lab, we will look at the microscopic nematode, *C. elegans.* These worms eat bacteria and you feed them bacteria that have been transformed with an RNAi molecule. This molecule degrades the production of collagen in the worms. Over three days time, this degradation of collagen will cause the worm to lose its shape and become short and fat, or "dumpy." This lab allows the students to make a visual connection between RNA, protein and phenotype. You can ask students: how did changing gene expression affect the microenvironment? What are the observable changes on a macro scale? What might happen to cells as a result of this drastically-changed microenvironment?

You must receive training on teaching this lab before picking up the kit. For more information, visit:

http://www.biobridge.us/index.php?option=com_content&view=article&id=296:rnai-and-genetics&catid=91:biobridge&Itemid=213.

AP extension
Cell behavior is similarly determined by signals from outside the cell. Example is cell migration (a property of stem cells as well as somatic cells such as fibroblasts.)
What determines the movement of a cell?

1. Cell invasions video: http://www.dnatube.com/video/1184/Cell-Invasions

Microenvironment and Cancer
Cells live in tissue (connective tissue and other cells). The microenvironment around the cell is anything to which the cell could potentially respond, typically by proliferating or differentiating, or by exhibiting a behavior. It also may keep cancer cells "in check." Lecture using supplementary Microenvironment PowerPoint slides (http://cirm.ca.gov/curriculum_unit-3) and information below.

I. Here are two examples of normal microenvironments:

1. View the animation Intestinal Crypt Stem Cells - A Clonal Conveyor Belt. By Eric Keller/Digizyme: http://www.molecularmovies.com/showcase/index.html#stemcells (click View the animation)

2. Hair follicles Examination and Web Exploration

    a. Examine the cells in the hair follicle using pictures from this site: http://biology.clc.uc.edu/fankhauser/Labs/Anatomy_&_Physiology/A&P201/Integumentary/Integument.htm

        i. Can also use prepared slides of hair follicles. (Buy slides from Wards, Fisher, etc.)

    b. Optional: Teach the different types of epithelial tissues and connective tissues using virtual labs.

    c. Discuss…what is the hair follicle microenvironment? Where are the stem cells housed and what are are their microenvironments (what cells are next to them, etc.)? What might be the result of changing this particular microenvironment? Ex. Hormones (testosterone, other androgens) in or near the hair follicle increases growth of hair. Can you think of any products that increase hair growth and how they might work? (Students could search for this information online.)

II. Aberrant gene expression combined with abnormal microenvironment = Cancer

   a. Cancer is caused by mutations in proto-oncogenes, resulting in oncogene expression (lecture from background information)

   b. There are different hypothesis to explain how cancer grows and spreads: examples are the Cancer Stem Cell hypothesis and the Clonal Evolution Model. Describe how the Cancer Stem Cell hypothesis differs from the Clonal Evolution Model. Refer to the diagram available here: http://www.landesbioscience.com/journals/cc/article/4914/. Click "Download PDF" to read the article; Figure 1 is a diagram visually comparing the two hypotheses.

Readings

Pick one of the following articles to read first.

1. EASY-MID: American Cancer Society article "Oncogenes and Tumor Suppressor Genes" http://www.cancer.org/docroot/ETO/content/ETO_1_4x_oncogenes_and_tumor_suppressor_genes.asp

2. MID-HARD: Nature SciTable article "Gene Expression Regulates Cell Differentiation" http://www.nature.com/scitable/topicpage/Proto-oncogenes-to-Oncogenes-to-Cancer-883

Then, read one or more of the following articles:

1. EASY LEAD-IN - Science Daily Article and Video: Unraveling brain tumors http://www.sciencedaily.com/videos/2007/0910-unraveling_brain_tumors.htm

2. EASY/MID – Researchers Find Prostate Cancer Stem Cell http://www.reuters.com/article/scienceNews/idUSTRE58857N20090909

3. EASY (Abstract) -- Dennis Disher, Tissue Cells Feel and Respond to the Stiffness of their Substrate http://www.sciencemag.org/cgi/content/abstract/310/5751/1139

4. MID – The Stem Cell Hypothesis http://www.crmagazine.org/archive/Breastcancer2008/Pages/TheStemCellHypothesis.aspx?Page=5

5. MID/CHALLENGING – Stem Cells May Be Key to Cancer, NY Times article http://www.nytimes.com/2006/02/21/health/21canc.html

6. (News Report) UCSD researchers pave the way for effective liver treatments http://www.eurekalert.org/pub_releases/2009-10/uoc--urp100909.php

7. MID – Diabetes Drug Selectively Kills Cancer Stem Cells In Combination Treatment in Mice http://www.sciencedaily.com/releases/2009/09/090914110530.htm

8. MID (News Report) -- Sean Morrison challenges cancer stem cell existence in melanoma http://www.hhmi.org/news/morrison20081204.html

9. CHALLENGING (Abstract with figure) Mattais Lutolf @ Stanford – hematopoietic stem cell ME http://www.rsc.org/Publishing/Journals/IB/article.asp?doi=b815718a
10. VERY CHALLENGING (the research paper from 7.) – Metformin Selectively Targets Cancer Stem Cells, and Acts Together with Chemotherapy to Block Tumor Growth and Prolong Remission http://cancerres.aacrjournals.org/cgi/content/abstract/69/19/7507

AP extension Discussion Question
Odd gene expression pattern causes cancer, but sometimes cells with these genetic mutations are not necessarily “cancerous,” i.e. they lack the phenotype of a cancer cell. What could be preventing them from doing so?

Application

1. Human Mammary Gland Progenitor Cells: First, understand the role of the microenvironment in normal development and cancer (lecture from Background Information section).
2. Then (optional), understand arrays *in general* by looking at cDNA array methods and data or other types of simpler array systems like Enzyme-linked Immunosorbent Assay (ELISA).
    a. Dolan DNA Learning Center – cDNA array animation
    http://www.dnalc.org/resources/animations/dnaarray.html
    OR
    http://www.bio.davidson.edu/Courses/genomics/chip/chip.html
    b. Microbiology, Prescott, Harley, and Klein — ELISA animation
    http://highered.mcgraw-hill.com/sites/0072556781/student_view0/chapter33/animation__quiz_1.html
3. Third, understand immunocytochemistry using fluorescently-tagged antibodies.
    a. Antibodies are an important research tool for cell biology. Scientists use antibodies generated in different animals to identify specific types of stem cells based on the different molecules those cells produce on their surfaces or inside their nuclei. To visualize a certain type of stem cell using fluorescence microscopy, a scientist would prepare a thin slice of tissue or a small dish of cells, then incubate the tissue or cells in what's called a "primary antibody" specific to one molecule on the surface of the stem cell of interest. This primary antibody recognizes only those molecules and attaches to them. Next, the scientist would incubate the tissue slice or cells in a "secondary antibody" fused to a fluorescent molecule. This secondary antibody only recognizes and binds to the primary antibody. Now, the scientist looks at it under a fluorescent microscope. The microscope uses a certain wavelength of light that excites the fluorescent molecules marking the stem cells of interest, causing them to emit another wavelength of light that can be detected by the microscope and your eye.
        i. EASIER - Immunofluorescence Labeling method
        http://www.bio.davidson.edu/COURSES/genomics/method/IMF.html
        ii. HARDER – Immunofluorescence Labeling of Cells, Sigma-Aldrich
        http://www.sigmaaldrich.com/life-science/cell-biology/antibodies/antibodies-application/protocols/immunofluorescence.html
        iii. Mammary gland pictures from LaBarge lab (Appendix C, cirm.ca.gov/curriculum_unit-3)

iv. Fluorescently-tagged stem cell pictures are available on CIRM's flickr page.
    1. http://www.flickr.com/photos/cirm

4. Understanding the Microenvironment Array (MEArray)
    a. Mammary progenitor cell: turns into myoepithelial cells or luminal epithelial cells, but what are the signals telling them to change??
        i. **LBNL News Story**: http://newscenter.lbl.gov/feature-stories/2009/02/24/it-takes-a-village-cell-microenvironments-hold-key-to-future-cancer-and-regenerative-medicine-therapies/
        ii. **AP biology extension:** Human mammary progenitor cell fate decisions are products of interactions with combinatorial microenvironments, read abstract and figure: http://www.rsc.org/publishing/journals/IB/article.asp?doi=b816472j
5. **Design your own MEArray research project:** Help your students describe how a scientist would test putative ME factors' effects on mammary gland progenitors using MEArray technology. See MEArray Teacher Guide (Appendix D, http://cirm.ca.gov/curriculum_unit-3) describing this project.

AP Extension questions: How do forces effect cell fate decisions? Can an ME Array test this? (No, it can only test ECM molecule- or signaling protein- interactions and cell-cell contact—not forces.) How would you test the effects of forces on stem or progenitor cells?

a. Short introduction: Growth factors, matrices, and forces combine and control stem cells. http://www.sciencemag.org/cgi/content/abstract/324/5935/1673 (abstract)
b. Cell fate determination research covers three areas:
    1. Adhesion-dependent cell survival
    2. Biomechanical force and the control of cell phenotype
    3. Adhesion-dependent manipulation of stem cell fate
c. For a MID/CHALLENGING overview of these areas: http://www.wtccmr.manchester.ac.uk/ourresearch/cellfatedetermination/index.asp
d. See work of Sanjay Kumar's lab, UC Berkeley Bioengineering Department http://kumarlab.berkeley.edu/
e. Articles about how forces affect cell fate
    i. EASY/press release: http://www.eurekalert.org/pub_releases/2008-09/uoc--ubb092008.php
    ii. MID: http://innovations.coe.berkeley.edu/vol1-issue1-fall07/cellularconnections
    iii. MID/CHALLENGING: http://kumarlab.berkeley.edu/biophysics.html
    iv. MID/CHALLENGING: http://kumarlab.berkeley.edu/colloidal.html

v. VERY CHALLENGING: http://kumarlab.berkeley.edu/publications.html, then scroll down and click the PDF link after this article:

J. Keung, K. E. Healy, S. Kumar, and D. V. Schaffer (2009). Biophysics and dynamics of natural and engineered stem cell microenvironments. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine.*

Assessment

Describe how a cell's *genotype* is converted into its *phenotype.*
What are the four components of the *microenvironment?*
What is cell behavior? Give three examples.
Give a specific example of how the microenvironment guides a cell fate decision.
Put the following terms in their proper sequence of appearance during gene expression: protein, splicing, mRNA, DNA, translation, pre-RNA, transcription, tRNA.
Define a stem cell niche and give one example. (Intestinal crypt or hair follicle)
Describe in general how a Microenvironment Array/cDNA array/ELISA works and why a scientist would want to use it.

AP thought questions (possible web research project to answer these)

- Is a genetic mutation necessary for cancer?
- Could cancer occur only by manipulating the microenvironment?
- If you took a piece of normal tissue and inserted it inside a tumor, what would happen?
- If you infect a chicken embryo with a cancer-causing virus, and the chicken grew up cancer-free, would you assume the chicken's cells were noncancerous?
- How might we test ways to see if certain microenvironments can stop cancer from growing?

Resources:

Science Daily Video: Unraveling brain tumors (COPYRIGHT *American Institute of Physics)*
http://www.sciencedaily.com/videos/2007/0910-unraveling_brain_tumors.htm

University of Utah: Interactive
http://learn.genetics.utah.edu/content/tech/stemcells/

Molecular Movies: Stem cells
http://www.molecularmovies.com/showcase/index.html#stemcells
LBNL News Story: http://newscenter.lbl.gov/feature-stories/2009/02/24/it-takes-a-village-cell-microenvironments-hold-key-to-future-cancer-and-regenerative-medicine-therapies/
LaBarge paper (Bissel Lab)
http://www.rsc.org/ej/IB/2009/b816472j.pdf

Detailed guide to making the MEArray
http://cat.ucsf.edu/resources/printManual_v2.3.5.pdf

Tumors and microenvironments:
http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WWK-4CWRRM1-6&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&_docanchor=&view=c&_searchStrId=1032872556&_rerunOrigin=scholar.google&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=4c180efce20e3ea2f495d84d953721af
AND
http://www.nature.com/nature/journal/v411/n6835/abs/411375a0.html